MEDICAL REVIEWS

Coronaviruses[1]

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INTRODUCTION

The coronaviruses are a group of RNA-containing agents which have been associated with respiratory illnesses in man and with a number of other conditions in laboratory and domestic animals. The name for the group was adopted to describe the characteristic fringe of crown-like projections seen around the viruses in electron microscopy; these projections are rounded, rather than sharp or pointed, as is the case with the myxoviruses. Like the myxoviruses, the coronaviruses contain essential lipid and are 80–160 nm in diameter (18). While the animal strains are readily isolated in several different systems, recovery of the human strains has posed major problems. A number of these strains have been isolated only in organ culture of the human respiratory tract. This factor has rendered difficult determination of the relationship between isolates and complicated efforts at understanding the role of these viruses in human respiratory illness. Therefore, much of the information on the epidemiology of the agents has come from serologic studies.

HISTORIC BACKGROUND

The first human coronaviruses were isolated by different techniques in the United States and Britain at approximately the same time. The British Medical Research Council's Common Cold Research Unit had been studying fluids collected from persons with natural respiratory infections both by standard cell culture isolation methods and by inoculating them into human volunteers. Rhinoviruses or other cytopathogenic agents could be recovered from a portion of the fluids (36). There was an additional substantial portion from which no agents could be isolated but which could still cause colds in the volunteers. Organ cultures of human embryonic trachea or nasal epithelium were then used in an effort at detecting the recalcitrant

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viruses present in the fluids. A specimen, B814, which had been collected in 1960 from a boy with a common cold had not yielded a virus on inoculation into cell culture. After the specimen had been passaged serially three times in human tracheal organ culture, it could still cause colds on inoculation into volunteers, which indicated that replication had taken place. When the specimen was passaged similarly in ferret tracheal culture it lost its ability to produce illness. Additional evidence of viral replication in human organ culture was interference with growth of a second challenge virus and some reduction in ciliary activity. (58)

In Chicago during the winter of 1962, five agents were isolated in primary human kidney cell cultures from specimens collected from medical students with common colds. The viruses were ultimately adapted to WI-38 cultures and exhibited a type of cytopathic effect not previously seen. A prototype strain, 229E, was selected for characterization and was found to be RNA-containing, ether labile and 89 nm in diameter, but distinct serologically from any known myxo- or paramyxoviruses. Sera collected from the five medical students all exhibited a fourfold rise in neutralizing antibody titer against 229E (23).

The fact that these "novel" viruses were of more than passing significance became clear when organ culture methods were added to standard cell culture techniques in a study of acute respiratory infections of adults conducted at the National Institutes of Health. Six viruses were found which grew in organ but not cell culture and were ether labile; on electron microscopy the agents were shown to resemble avian infectious bronchitis virus in structure (39). The B814 and 229E strains were soon also demonstrated to have a similar structure on electron microscopy and to develop in infected cells by budding into cytoplasmic vesicles (1, 2, 22). As a result of the similarity of the human agents to infectious bronchitis virus (IBV) and also to mouse hepatitis virus (MHV), they were collectively considered to represent a group of vertebrate viruses distinct from the myxoviruses antigenically and structurally (3). The name coronavirus was adopted for the group to describe the fringe of projections seen around them on electron microscopy (18).

Except for 229E, none of the human coronaviruses had been successfully propagated in a system other than organ culture. McIntosh et al. reported successful adaptation of two of the NIH isolates, OC (organ culture) 38 and OC43 to the brains of suckling mice. These strains were shown to be essentially identical antigenically but quite distinct from mouse hepatitis virus. Only OC38 and OC43 could be so adapted; the other four OC strains resisted such attempts (37). Infectious bronchitis virus was known to exhibit hemagglutination under certain conditions but no such phenomenon had been demonstrated for the human strains until OC38 and OC43 had been adapted to mice. Kaye and Dowdle found that the infected brain preparations would directly agglutinate red cells obtained from chickens, rats and mice. The agglutination was specific and was not inhibited by receptor-destroying enzyme. This technique greatly expanded the ability to do epidemiologic studies since it was simple and reproducible (32).

Other more recent developments have included adaptation of OC38 and OC43 to growth cell monolayers. Either mouse brain or organ culture material could be used as source of virus; CPE appeared in tube cultures of primary rhesus or vervet monkey kidney cells after two to three passages (11). Further adaptation to other cell systems was also possible; cytopathic effects developed in BS-C-1 cells by about seven days after inoculation. Not only was cytopathic effect available for reading of neutralization tests; the OC38-43 virus was found to hemadsorb

red cells of rats and mice, making available a more precise means of evaluating endpoints in tests involving these organ culture derived strains (30). The other OC strains which could not be adapted to mouse brain resisted adaptation to cell culture. Finally, immune electron microscopy has been added to the methods available for identifying the presence of coronaviruses in organ culture harvests. This highly sensitive technique should improve the ability to detect virus, but is obviously not suitable for use in all but the most specialized studies (31).

METHODOLOGY

Sources of Mortality Data

Coronaviruses that infect domestic and laboratory animals produce illnesses which are sometimes fatal. In contrast, there is no documented report yet on record of human coronaviruses being involved in a lethal infection. This situation may be a reflection of the limited number of investigations carried out as yet. It is known that these agents frequently infect small children and reinfect adults, including persons with chronic respiratory disease (53). It would be logical to assume that deaths could occasionally occur in these most susceptible segments of the population, but they are probably not very frequent.

Sources of Morbidity Data

Since coronaviruses usually produce respiratory illnesses indistinguishable from those caused by many other types of viruses, it is not possible to obtain data on morbidity in the absence of laboratory identification of infection. The viruses are difficult to isolate, so that most workers have relied on serologic techniques to increase the numbers that can be studied. Investigations into coronavirus infection have usually formed part of overall evaluations of the role of viruses in general in respiratory illnesses. As indicated in the partial listing in Table 1, a variety of different open and closed populations have been used for these studies. The 229E strain was originally isolated from medical students in Chicago as part of a long-term study of respiratory illnesses in young adults (21, 23). Employee groups have been the source of specimens in the National Institutes of Health (29, 42) and in the studies at Charlottesville, Virginia (26). Infection has also been evaluated in children's homes (34) or boarding schools (Kendall) (36) in the military (60), and among children hospitalized for severe respiratory illnesses in various parts of the world (29). Serologic methods have been used to detect occurrence in persons with acute exacerbations of asthma (40) or chronic obstructive respiratory

TABLE 1

LONGITUDINAL STUDIES ON THE EPIDEMIOLOGY OF CORONAVIRUS INFECTION IN HUMANS

Location	Population	Virus studied
Chicago, IL (21)	Medical students	229E
Washington, DC (29, 42)	Hospitalized children	229E, OC43
Bethesda, MD (29, 42)	Adult employees	229E, OC viruses'
Atlanta GA (34)	Institutionalized children	OC43
Charlottesville, VA (26)	Working adults	229E, OC43
Tecumesh, MI (16, 49)	General community	229E, OC43
Brazil (15)	Non-hospitalized children	229E
Denver, CO (40)	Hospitalized asthmatic children	229E, OC43
N. and S. Carolina (60)	Military	229E, OC43

disease (53). Patterns of coronavirus infection have been identified among the general population residing in the Tecumseh community as part of a longitudinal study of respiratory illness (16, 49). Human volunteers have continued to be employed, especially to determine characteristics of illness not yet well defined in natural infection because of problems associated with isolation of the viruses (7, 8).

Serologic Surveys

Although relatively simple serologic techniques are now available for two types of coronaviruses, extensive surveys of antibody prevalence have not been carried out. When done, the surveys have often formed a part of studies mainly directed toward determination of incidence of infection. Information on prevalence of antibody is available for populations in the United States, (16, 26, 42) Britain (8), and Brazil (15). A special situation is the presence in man of antibody against two coronaviruses of animals. The finding of mouse hepatitis antibodies in military recruits and in children and adults from the general population was surprising when first described in 1964 (25). It is now recognized that this does not indicate past experience with MHV, but rather with human coronavirus strains which are known to cross react with it. In contrast, in a survey of antibodies to avian infectious bronchitis virus, none could be found in a military population. Low level antibodies were detected only in a portion of individuals who had close contact with poultry (45). This virus is not known to cross react with the human strains.

Laboratory Methods

Isolation

Only the 229E strain was originally isolated in cell culture. It was eventually adapted to WI-38 cells, in which it has been maintained (23). However, this cell line is not a reliable system for primary isolation of 229E-like agents. To date, human embryonic intestine (MA177) has proven the most suitable cell system but it is available only in limited quantities (29). All other known human coronaviruses were originally isolated in organ cultures of human trachea or lung (24, 39, 58, 59). The presence of virus was usually detected by electron microscopy, or sometimes by fluorescent antibody testing of impression smears (57). Two strains, which are essentially identical, (OC 38 and 43), have been adapted to mouse brain and to primary monkey kidney and BS-C-1 cell cultures (11, 30, 37). Another cell system L132, a heteroploid human lung line, has been reported to be suitable for primary isolation of 229E, a related virus (LP) and the first described organ culture agent B814 (6, 9). This latter finding has not been confirmed by other workers (11).

Serologic tests

Neutralization tests of varying degrees of complexity can be performed for all described coronavirus types. The most involved procedure must be used for those viruses which up to now have never been adapted to systems other than organ culture (39). This technique involves incubating serum with known virus and inoculating the mixture into cultures of human trachea. Reduction in viral yield is determined by electron microscopy. For those viruses adapted to cell cultures, tube or plaque reduction neutralization tests are available. WI-38 or L132 cells may be used for both methods in tests involving 229E virus; a number of cell lines including primary monkey kidney and BS-C-1 have been used for neutralization

tests involving the OC38–43 virus (5, 9, 11). Hemadsorption rather than cytopathic effect can be used for identification of endpoints with the latter cell line (12, 13).

Most of the seroepidemiologic studies have not used neutralization but rather complement-fixation (CF) or hemagglutination-inhibition (HI) tests as sources of their data. The method of preparing a CF antigen for 229E directly from cell culture harvests was reported along with the original description of the viruses by Hamre and Procknow (23). The antibody detected was of low titer and appeared to be present for only a short time after infection. This observation was subsequently confirmed in a large scale study, and it was suggested that the presence of CF antibody in a population could be interpreted as evidence for recent activity of the virus (15). However, if the antigen was highly concentrated, antibody could be detected at a higher titer, and this antibody persisted in the population so that the method could be employed in surveys of prevalence (5). An indirect HI test has also been described for 229E virus using tanned sheep erythrocytes. The procedure appears to be highly sensitive and no cross reactions with OC43 were observed (35).

Complement-fixation tests can be performed with OC38–43 virus using infected suckling mouse brain as antigen. Antibody response of individuals infected with this virus appeared to be specific enough to avoid confusion with 229E (42). The same mouse brain material could also be used for the HI test of OC38–43 antibody. The hemagglutination titer was higher for rat than chicken erythrocytes, but was sufficient with the chicken cells so that they could generally be employed in HI tests; this is of particular importance in view of spontaneous agglutination which often complicates working with rat erythrocytes. Serum to be tested did not require treatment with receptor-destroying enzyme but rather standard heat inactivation at 56°C. The agglutination took place equally at various temperatures, including room temperature (32).

Other serologic tests have been developed which have been used more in antigenic analyses of the different coronaviruses than in epidemiologic studies. With the indirect fluorescent antibody technique, characteristic cytoplasmic inclusions were demonstrated with 229E, OC43 and even with the other viruses grown in organ culture and prepared for testing by making smears of fragments of the infected trachea (41). It has also been possible to demonstrate precipitin lines on gel diffusion tests with coronavirus antigens concentrated 10- to 50-fold. Two or three precipitin lines were observed by Bradburne in tests with hyperimmune animal or human serum (5), but others have identified only one such line (33).

BIOLOGIC CHARACTERISTICS OF THE VIRUS

The coronaviruses of animals produce serious illnesses in their respective species. In addition to hepatitis of mice and infectious bronchitis of chickens these viruses are responsible for transmissible gastroenteritis and encephalomyelitis in pigs and neonatal diarrhea in calves (44, 55, 56). They are rather species-specific in their *in vitro* growth characteristics, especially on primary isolation, but such isolation is easily accomplished (54). In contrast, the human coronaviruses produce relatively superficial infections of the respiratory tract. Species-specificity is observed here too but even in cell systems of human origin, isolation of some types has not been possible. It is conceivable that special conditions are required for propagation of these agents; this would be similar to the situation that obtained with the rhinoviruses, another surface pathogen, before the availability of WI-38 cells (52).

Very little information is available in the relationship of coronavirus structure to patterns of infectivity and antigenicity. The viruses resemble the myxoviruses in size, type of nucleic acid contained, and, to a certain extent, in morphology. The spikes of the virus are distinct in appearance from those of the myxoviruses and are reported to contain glycopolypeptides; both HA and CF antigens have been associated with the surface of the virion, and are presumably located in the projections. No neuraminidase has been demonstrated, and therefore, it has been concluded that the antigens belong to a single species present on the surface. By analogy, antibodies to these antigens should be associated with protection (28, 33).

The total number of serologic types infecting man has not been defined. The problem here again revolves around the difficulties encounterd in isolating the human coronaviruses. There is no way of estimating the proportion of existing types that have already been isolated. It is also difficult to determine the separate antigenic identity of types which grow only in organ culture as compared with those which grow in cell culture. Neutralization, CF, HI, gel diffusion and immunofluorescent techniques have been used in the antigenic analyses by McIntosh et al., Bradburne, and Bradburne and Somerset (41, 5, 8). As would be expected, results have differed by each of these procedures, with neutralization tests the most specific. However, cross reactions were commonly demonstrable even by this method using animal antiserum or immune ascitic fluid, indicating that there must be many shared antigens. An attempt at placing the human coronavirus in broad groups is shown in Table 2; mouse hepatitis virus (MHV) is included because of its frequent interrelationships with the human strains and avian infectious bronchitis virus omitted because it is antigenically distinct. The unadapted organ culture strains have been listed separately; it has not been possible to prepare animal antisera against them, and they have been tested only against pairs of serum obtained either from individuals naturally infected or volunteers challenged artificially. Such sera would be expected to be considerably less specific than animal antisera. It has been clearly shown in several laboratories that 229E is quite different from OC38 and OC43 not only in growth characteristics, but also antigenically; cross reactions can be shown by neutralization tests but these are demonstrable only using very sensitive procedures. The LP virus was originally isolated in organ culture and not in cell culture but is closely related antigenically to 229E. The OC38–43 virus has a low

TABLE 2

SEROLOGIC RELATIONSHIPS OF THE HUMAN CORONAVIRUSES

Group	Strains tested with animal antisera	Strains tested with human antisera
I	229E ⎫ Closely related but LP ⎭ not identical	
II	OC38 ⎫ Nearly identical OC43 ⎭ MHV	OC44
III	B814	
Others		OC16 OC37 OC48 EVS

level cross reaction with mouse hepatitis virus; in some reports this has been reciprocal and in some one way. Although B814 virus is quite different from OC38–43 virus they both share some antigens in common; again cross reactions with 229E are rare. Among the additional viruses, OC44 is closely related antigenically to OC38 and OC43 but has never successfully been adapted to mouse brain or cell cultures. The four other viruses are listed together by exclusion, not because of any demonstrated relationship to one another, but rather because they are not closely related to viruses in the first three groups. Some low level reactions with the agents in these three groups have been shown to be present, with OC16 virus being the most distinctly different strain.

While neutralization methods are of value in grouping virus, the CF and HI tests and their specificity are of special importance in planning epidemiologic studies; these tests are generally available only for the 229E and OC38–43 viruses. Cross reactions between these two viruses have only rarely been reported when tested by CF against animal sera. With human serum, heterologous rises in antibody titer have been observed occasionally, but not frequently enough to create problems in studies involving significant numbers of specimens (10). Of greater practical concern is the occurrence of cross reactions between OC38–43 and the other organ culture viruses. It is possible that rises in titer detected when using OC43 antigen in a seroepidemiologic study may have been caused either by OC43 infection itself or by infection with one of these other viruses which share antigens with it. Indirect evidence that the infecting agent may not be OC43 itself is the apparent dissociation between the CF and HI tests for OC43 observed during a particular period of time. Rises in titer by CF should usually be accompanied by rises in titer by HI in the same serum pairs. If this does not ordinarily occur during one time period but does during a second period, it indicates that a related virus, but not OC43 was circulating during the first time (49).

Data on the etiologic role of coronaviruses in respiratory infections derive from laboratory and field studies. The viruses do interfere with the action of cilia in tracheal organ culture, which suggests that they should have the same effect *in vivo*. In addition, volunteers have been inoculated with essentially all available strains with production of illness (7, 8). It has also been possible with 229E to demonstrate that natural infecton was statistically related to the production of illness. During the 1967 outbreak of 229E infection in Tecumseh, MI, illness was significantly more common among those with infection than among matched individuals without infection (16). Similarly, 229E infection among Chicago medical students was statistically associated with illness when those with rises in titer were used as their own controls (21).

DESCRIPTIVE EPIDEMIOLOGY

Incidence and Prevalence

Evidence is steadily mounting that the coronaviruses are of major importance in common respiratory infection of all age groups, especially those occurring in midwinter and early spring. The total impact of coronavirus infections on the general population cannot be calculated at present because not all viral types have been identified. Only 229E and OC43 are amenable to large scale serologic studies; infection rates for other distinct types such as OC16, cannot be determined. The assumption must be made that the former two types are typical of the other viruses.

Incidence of infection with these agents exhibits a marked cyclic pattern so that it is to be expected that reported rates will vary based on the number of seasons of high viral activity included in a particular study.

Incidence and Prevalence of 229E Virus

The activity of 229E was found to be of high prevalence in three out of six years of a study among Chicago medical students. The mean annual incidence of infection during the total period was 15%, based on person-years of observation. The criterion for identification was a reproducible twofold seroconversion determined by CF. There was marked year-to-year variation in infection frequency, ranging from a high of 35% of those tested in 1966–1967 to a low of 1% in 1964–1965. However, nearly 97% of the infections occurred during the months from January to May, often at a time when isolation of rhinoviruses was at a low, and seroconversions for 229E were only rarely accompanied by a rise in titer for another respiratory agent (21).

The serologic study of 229E activity in the community of Tecumseh, Michigan initially covered two years, which included one period of high prevalence. Like the study in Chicago, routine blood specimens were collected so infection rates could be determined; unlike it, the study group was composed of individuals of all ages living in their homes. Over the two years, infections were detected in 7.7% of individuals tested by CF, as shown in the curve in Fig. 1. However, this appeared to be an underestimate of the actual activity of the virus. Serum specimens had been collected on a regular basis, six months apart; rises in titer by CF occurred most frequently in those pairs in which the second specimen was collected in April 1967, clearly indicating the peak period of viral dissemination. At this time, the neutralization test was moderately more sensitive than the CF procedure. However, because of the brief persistence of elevated CF antibody following infections, the CF test became much less sensitive for testing sera that were collected after April. Here the neutralization test could still be relied upon. Therefore, CF and neutraliza-
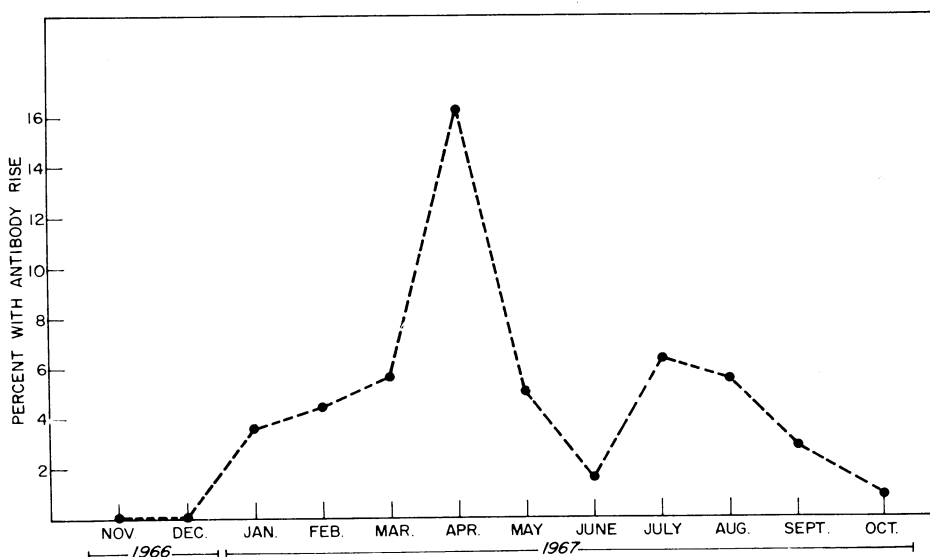

FIG. 1. Serologic incidence by CF of infection with 229E virus in Tecumseh, MI, 1966–1967.

tion test results were combined to give an overall infection rate for the population studied; this rate, 34%, was remarkably similar to the 35% observed in Chicago at the same time. Because of the limited period of viral activity it was possible to compare illness rates of those infected with persons not infected matched by age and sex; it was estimated that 45% of the infections had produced clinical disease. Thus in the population, the rate of 229E-associated illnesses during the outbreak was 15 per 100 persons studied. Clustering of infections in family group-ings were apparent as was activity in all age groups, including children under five years of age (16).

In other investigations of 229E activity, attention has been directed mainly to-ward study of associated illnesses; in such studies, sera have been collected before and after the illness, rather than continually on a routine basis as done to determine infection rates. Employees at State Farm Insurance Co. in Charlottesville, VA were studied during an eight-year period for rises in titer for both 229E for OC43. By CF, 229E infection could be related to 3% of the colds that occurred in the winter-spring and to 0.4% of colds that occurred in the summer-fall. While there was some year-to-year variation in activity, differences in the number of specimens tested from various years did not permit complete identification of cyclic patterns. In particular, no specimens were available for the 1966–1967 winter-spring season (26). Employees of the National Institutes of Health with respiratory illness were studied by both isolation and serology for 229E infection over a six-year period. Again attention was specifically directed toward certain segments of the six years, and no specimens were tested during other segments. Of particular interest once more is the segment from December 1966 to April 1967. Isolation of rhinoviruses and myxoviruses were uncommon at this time, but respiratory illnesses continued to occur. During that period, 24% of those persons with colds studied had rises in titer for 229E. As part of the same investigation, paired blood specimens col-lected from infants and children admitted to hospital with acute lower respiratory disease during the 1967 period of 229E activity were tested for rise in antibody against the virus but none was found (29, 42).

Surveys of prevalence of 229E antibody have also been carried out to document past history of infection, often as parts of longitudinal studies. A general finding is that antibody is present in a significant portion of adults who, in spite of posses-sing this antibody, can go on to have reinfection and illness. Reports of antibody prevalence in adults in the United States have varied from 19 to 41%, depending on the type of test used to determine antibody and time of collection of serum (16, 26, 42). Children under 10 years of age exhibited lower mean antibody titers than older children or adults (16, 42). Individual sera from normal healthy adults collected serially in Britain from 1965 through 1970 were tested by Bradburne and Somerset (8). It is of interest that there was a buildup in sera positive by CF from approximately 17% in specimens collected in October–December 1966 to 62% in those collected in July–September 1967. This would suggest that the spring 1967 outbreak which occurred in several parts of the United States may have taken place in Britain as well.

Incidence and Prevalence of OC43 Virus

Populations employed to study infection and illness caused by OC43 virus have generally been the same ones employed to study the occurrence of 229E virus. Kay *et al.* used an additional group, healthy children institutionalized in Atlanta,

GA, in which to identify infection by means of their HI test. The investigation, carried out from 1960–1967, involved collection of serum specimens related to illness and also routine collection of sera from some non-ill individuals. Infections with the agent were detected in all years of the study but with definite cyclical variation. Seasons most involved were the winter and spring. Overall, 3% of the illnesses recorded in the seven years could be associated with OC43 infection, with a high of 7% in 1960–1961. Interestingly, testing of the sera collected routinely from non-ill individuals indicated that an additional equal number of OC43 infections were occurring without the production of symptoms (34). The Charlottesville study of adult employees studied OC43 infections along with 229E. Here too the emphasis was on illness and it was found in all years studied that OC43 as associated with 5% of colds in the winter–spring and with no illnesses in the summer–fall. Again there was cyclical variation from year to year in the number of rises in titer detected (26).

The original isolations of OC38 and OC43 were made in December and January 1965–1966 as part of the study carried out among NIH employees with colds. Testing of sera collected from these employees indicated that during this period up to 29% of the colds studied were accompanied by rise in titer for OC43. In the children hospitalized with lower respiratory disease up to 10% of illnesses during this period were associated with such a titer rise. However, it was impossible to show that the relationship to disease was truly etiologic. This finding was in contrast to that seen with 229E in which no rises in titer were detected in such cases (42, 38). Another suggestion that OC43 might be involved in lower tract disease came from the study of coronavirus infection in acute respiratory disease (ARD) in the military. During a sharp outbreak of ARD in the winter of 1970–1971, many of the recruits had infections with other agents besides OC43. However, in 29.7%, there was evidence of only OC43 infection (60).

In the Tecumseh study, occurrence of OC43 infection was determined in the community population over a four-year period. CF and HI tests were used on all specimens and neutralization tests were used as an aid in evaluating these results in selected specimens. During the total period, OC43-related infection was detected in 17.1% of the 910 persons studied for one year. Most of the infections took place in the winter–spring months of 1965–1966, 1967–1968, and 1968–1969. The only winter-spring period without such activity was in 1966–1967 when the 229E outbreak had taken place. There was good agreement between the CF and HI tests for the 1965–1966 and the 1968–1969 periods but not for 1967–1968. The neutralization test was used to clarify the situation. It was found that most rises in titer for the periods in 1965–1966 or 1968–1969, whether they had occurred by CF or HI or both, were also accompanied by rises in neutralizing antibody. In 1967–1968, most CF rises in titer were not accompanied by rises in titer in the other test, nor was the reverse true; significant change in neutralizing antibody in this period was exceedingly rare. It was concluded that the outbreaks of infection in 1965–1966 and 1968–1969 probably were caused by agents closely related to OC43, while the 1967–1968 activity was due to one of the other OC viruses which shares some antigens with OC43 but is more distantly related to it. The 1968–1969 outbreak of OC43 infection was nearly as widespread as the prior 229E outbreak, with 25.6% of the population studied showing evidence of infection. Of special interest was the fact that children under five years of age had the highest infection rates (49).

Surveys of antibody prevalence have been conducted in several settings using OC43 antigens. McIntosh *et al.* found that children began to acquire antibody to this virus in the first year of life. By the third year of life, more than 50% had antibody present. Among adults, 69% of individuals could be demonstrated to have antibody; this indicates, in view of the high incidence of infection with the agents in all age groups, the frequency with which such infections must represent reinfection (42). The high prevalence of antibody has been confirmed in other studies (26, 34). Bradburne and Somerset followed prevalence of antibody for OC43 over time, as they also had done with 229E. Each year the greatest prevalence of antibody was found in the winter–spring period. The single highest point in antibody prevalence was in January–March 1969, at the same time the OC43 outbreak was occurring in some parts of the United States (8).

Geographic Distribution

Occurrence of coronaviruses infection has been documented, either by isolation or serology, from coast to coast in the United States. In addition to the studies listed in Table I a 229E-like virus has been isolated in California and OC43 and 229E have been demonstrated to be present in Vermont by serologic methods. (51, 53). Extensive studies have, of course, been carried out by the Common Cold Research Unit which has demonstrated the presence of the agents in Britain. The activity of 229E virus has been documented in Brazil in a study of children and adults with and without respiratory illness. Significant rises in antibody titer accompanied non-hospitalized respiratory infection in the children. Prevalence of antibody was determined by CF, and like the situation in some studies in the North Temperate Zone, children had little antibody while 26% of adults were antibody positive (15). These findings suggest that coronaviruses will be found to be worldwide in distribution and to cause similar types of illness in different localities; such a situation has been noted with many other respiratory viruses (47). An attempt was actually made to detect rises in antibody titer for 229E in paired sera collected from small children with lower respiratory infection in many tropical parts of the world. No evidence of infection was found, which is hardly surprising, since no rises in titer were found in similar sera collected as part of the same study in Washington, DC (17, 29).

Epidemic Behavior and Temporal Distribution

Because most illnesses caused by coronaviruses are similar to those caused by other respiratory viruses, it is impossible to identify epidemic behavior of the viruses. There is, however, great variation in the frequency of infection both on a seasonal and on a cyclical basis. Isolation and rises in antibody titer for all types of coronaviruses have been rare events outside of the period from December through May. This is the portion of the year in which isolation rates for rhino- and other respiratory viruses often reach their low. In addition, a cyclic pattern can be discerned when individual virus types are considered. In Fig. 2 are summarized data from five longitudinal studies of coronavirus activity carried out in different parts of the United States. In all studies some sporadic activity did occur in nearly all years studied, but rises in antibody titer were concentrated in certain years which far exceeded the means for the entire studies. Those periods are indicated as darker areas in the figure. The times during which specimens were collected in each investigation are indicated in the figure by the large rectangle. For 229E,
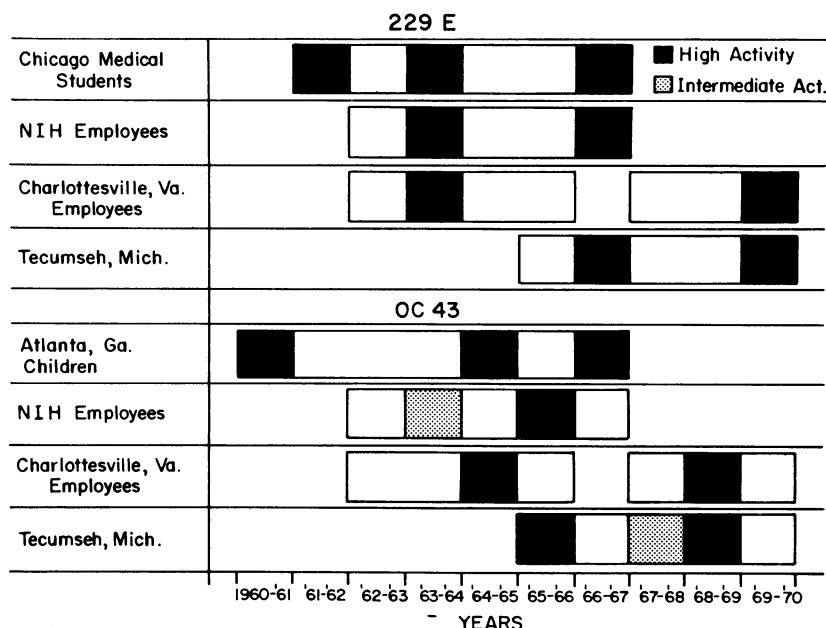
Fig. 2. Cyclic behavior of 229E and OC43 viruses observed in five longitudinal studies.

activity was detected in all four studies at the same times, even though two were in the Midwest and two in the Eastern United States. It seems possible, on the basis of these data, to postulate a two to three year cycle for this agent. The greatest number of infections in Chicago was seen in 1967, after absence of the agent for three years, which would suggest a role of herd immunity in determining the time of reappearance of the agent.

With OC43 the situation is quite different. As with 229E, in no investigation did two years with high rates of infection or illness follow one another. A possible exception was in the Tecumseh study. However, the agent which caused the rises in titer in 1967–68 did not appear as closely related serologically to OC43 as the agent involved in the other two outbreaks. This observation indicates a problem in identifying cycling of OC43. The virus undoubtedly shares more antigens with other identified or perhaps unidentified coronaviruses than does 229E (see Table 2) and these other viruses may well have cycles of their own which may confuse the situation. In 1964–65 high activity occurred in Atlanta and Charlottesville. However, in Bethesda, just a short distance away, high activity was not seen in that year but in 1965–66, the same time as high activity occurred in Michigan, many miles away. In 1968–69, Charlottesville and Tecumseh data did agree with very high activity in both areas. Thus cycling of the agents was found in all studies but they did not agree on specific years. This may be a result of actually different patterns of occurrence or because of differences in the serologic techniques used to identify infection, which are of greater importance with OC43 because of the problem of cross reactions. The fact that cycling of coronaviruses does exist and occurs every two to four years with production of many infections, suggests that the number of truly different coronaviruses must be relatively small, unlike the situation with the rhinoviruses, in which cycling has been more difficult to demonstrate, in part because of the large number of serotypes (14).

Age

All age groups are involved in infection with OC43 virus. High rates have been noted in children or adults during studies separately examining both groups. In the Tecumseh study, a total population group was followed. During the 1968–1969 outbreak, infection rates were relatively uniform for all age groups, varying from a high of 29.2 per hundred person-years in the 0–4 age group to 22.2 in those over 40 years of age (49). This finding is quite different from the situation that exists with other respiratory agents, such as respiratory syncytial virus, where a more distinct decrease in infection rates can be observed with increase in age (48). The reversal of the pattern of age-specific infection rates customarily associated with the respiratory viruses becomes complete with 229E. Infection has been more difficult to demonstrate in small children with this agent than in adults. In Tecumseh, during the 1966–1967 outbreak, highest age-specific infection rates by CF were found among those 15–29 years of age, following a steady increase in infection frequency from the 0–4 year olds. However, when neutralization tests were used to detect rise in antibody titer, the 15–19 year olds still had high infection rates; but the serial increase to that point among younger age groups was much less steep (16). This would suggest that the apparent sparing of small children with 229E may be a result of the relative insensitivity in the young of the serologic procedures commonly employed. It would be surprising if two different coronavirus serotypes behaved so differently.

Other Factors

There is little evidence of a sex differential in infections with the coronaviruses simply because the data have rarely been examined in such a manner. In Tecumseh, adult females experienced higher infection rates with OC43 than adult males, which is in conformity with the usual patterns of all respiratory illnesses (46). In the study of antibody prevalence of Cardeias, the results were examined by sex but no significant differences could be observed (15). There are no available data on occupation or racial susceptibility to infection nor on the role of socioeconomic status in influencing rates. Occurrence of infection in closed or special populations, such as the military or children's institutions has been reported (34, 36, 60). However, it is at present difficult to determine, based on the relative paucity of information on the behavior of the virus in open populations, whether they exhibit any unique features in other settings. The suggestion that OC43 virus might cause ARD in recruits, if confirmed, will be a distinct departure from the types of illness customarily associated with that virus in young civilian adults. The role of the school age child in dissemination of coronavirus has not yet been clearly defined, but it would be surprising if these infections differed in their transmission patterns so markedly from that documented with the other agents. Because of the high frequency of infection in older children and adults, other sites of dissemination may also be of significance. It has, actually, been possible to show that the family unit is of importance in transmission since clustering of 229E and OC43 infections in families was observed in the Tecumseh study.

While nutritional and genetic factors have not been associated with susceptibility to coronavirus infections, there are clear indications that the viruses are associated with exacerbations of chronic obstructive respiratory disease. Such a finding is hardly surprising in view of the high infection rates which have been observed

in unselected older adults. It has not as yet been demonstrated that this represents a true increased susceptibility to infection, or simply a more severe form of expression of the infection when it occurs in an already compromised host. In addition to the situation in older individuals, there is evidence that both OC43 and 229E may trigger acute attacks of wheezing in young asthmatics (40, 53).

MECHANISMS AND ROUTES OF TRANSMISSION

The coronaviruses are transmitted by the respiratory route. It has been possible artificially to induce infection in human volunteers by inoculating virus into the nose (7, 59). No other route of transmission for coronaviruses seems involved in man, although animal coronaviruses are infectious by the fecal–oral route (56). There is no direct evidence to aid in identifying the main mechanisms of transmission. However, it is possible to compare the epidemiologic behavior of the coronaviruses with other respiratory agents whose transmission mechanisms have been more directly studied. Large scale outbreaks of coronavirus infections have taken place, as in Tecumseh in 1967 (16). This is much more analogous to the situation seen with influenza than it is with the rhinoviruses. It is likely that the former agent can be transmitted by aerosol in addition to large droplet, which would explain its ability to spread quickly (19). Rhinoviruses on the other hand are transmitted by large droplet and at times may spread via fomites (27), but not by aerosol. It is therefore probable that human coronaviruses can be spread by aerosol as well as by large droplet. Aerosol transmission has actually been documented in poultry by the agent of avian infectious bronchitis (20).

There is no evidence that any animal reservoir or vectors are involved in the maintenance of infection or transmission of the human coronaviruses. Each animal coronavirus appears to be restricted to its own species. The only known exception is the finding of antibody of avian infectious bronchitis virus in sera of poultry workers, but not of controls (45).

PATHOGENESIS AND IMMUNITY

The incubation period of coronavirus colds is relatively short. In studies involving human volunteers, the mean period from inoculation of virus to development of symptoms was from 3.2–3.5 days depending on the strain, with a range of from 2 to 4 days (7, 59). Following exposure, the virus apparently multiplies superficially in the respiratory tract in a manner similar to that in which multiplication occurs in vitro. Virus excretion usually reaches a detectable level at the time symptoms begin, and lasts for 1 to 4 days. The duration of the illness is from 6 to 7 days on the average, but with some lasting up to 18 days. Serologic response to either induced, or to naturally acquired infection has been quite variable depending on the infecting strain and the serologic test employed. For example, among those experimentally infected with OC38–43 virus who had a cold produced, only 46% had rises in titer by HI and 23% by CF. Less than half of those infected with 229E showed a CF rise. It is not clear how the existence or titer of preinfection antibody affects the magnitude of the response detected by these tests. Rises in neutralizing antibody titer are much easier to detect, and have been found with sensitive techniques to be demonstrable in all experimentally infected (5, 8).

Many of the infections observed with the coronaviruses are in fact reinfections. In the Tecumseh study, 81.5% of those infected with OC43 actually possessed prior neutralizing antibody (49). Possession of circulating OC43 HI antibody

among the Atlanta children did not appear to play a role in modifying expression of the subsequent infection (34). With 229E virus, Hamre and Beam demonstrated that frequency of rises in titers detected by neutralization was inversely proportional to prior neutralizing antibody, which would indicate that this antibody exerted some protective effect. However, the importance of this neutralizing antibody could not be confirmed when infection was detected by CF (21). Thus, circulating neutralizing antibody as presently measured may bear a relationship to modification of infection but this association is not a very strong one. Since coronavirus infections mainly involve the surface of the respiratory tract, it is likely that secretory IgA antibody plays a more direct role in protection; this has in fact been demonstrated with a swine coronavirus (4).

PATTERNS OF HOST RESPONSE

The coronaviruses generally produce a cold-like illness which on an individual basis would be difficult to distinguish from those caused by other respiratory viruses. In both induced and natural infections the most prominent findings have been coryza and nasal discharge, with the discharge being more profuse than that customarily seen with rhinovirus colds. Sore throat has been somewhat less common, and in children has been associated with pharyngeal injection (34). Experimental colds caused by B814 virus were about as severe as those caused by 229E; however, natural OC43 infections caused illnesses with considerably more cough and sore throat than did 229E infections (26). The mean duration of coronavirus colds, at 6.5 days, is shorter than that seen in rhinovirus colds at 9.5 days (7). There is no clear evidence that these viruses cause severe lower respiratory illness in infants and young children. In fact, such infections were more common in one study among the control group than among the diseased (42). Mufson *et al.* have associated coronavirus 229E and OC43 infection with acute lower respiratory infections in children at Cook County Hospital (50). The lack of a comparable control group makes assignment of an etiologic role to the viruses hazardous at present, but the relationship should be sought in the future when it is known that outbreaks are taking place in the community. The association of OC43 with the acute respiratory disease (ARD) syndrome in military recruits should also be viewed as tentative.

Clinical disease occurred in no more than 45% of those infected with 229E in Tecumseh during the 1967 outbreak (16). In Atlanta children, OC43 virus produced illness in about 50% of those infected (34). It is likely that with increase in age and concomitant experience with these agents, the ratio of clinically apparent to inapparent infection will decrease. As with other respiratory agents, a continuum of severity of symptoms exists among those in which infection results in disease, and this may also be related to past experience with the viruses.

CONTROL AND PREVENTION

It is premature at present to think in terms of control of coronavirus infection. Not all viral types have been identified and some known agents cannot be easily propagated in the laboratory. Thus, preparation of vaccines of the conventional types is impossible. The frequency of reinfection observed with these agents is so high that control by vaccination may not be practical, but it is possible that further studies may allow identification of truly protective antibodies. There remains environmental control of infection; such efforts have only rarely been useful for other

respiratory agents and they are likely not to be more efficacious for the coronaviruses (43).

UNRESOLVED PROBLEMS

The major immediate need in coronavirus research lies in the laboratory. If a practical system can be found for isolation and propagation of the viruses, the gaps in understanding the behavior of these agents would quickly be filled. Only serologic tools are available now for most epidemiologic studies, and even these can only be applied to two different coronavirus types. Therefore much of the data which have been so laboriously gained gives only partial evidence on the total dimensions of the problem. And the problem is almost certainly a very large one. Coronaviruses have been isolated and outbreaks identified in periods of the winter and spring when rhinoviruses and myxoviruses are uncommon. It appears that during these times the coronaviruses cause a significant portion of respiratory illnesses. Even discounting suggestions of production of severe disease in young children and those with chronic respiratory disease, the viruses are important pathogens simply in terms of numbers of illnesses produced. Only through further understanding of the behavior of these agents will it be possible to determine by what means control can be attempted.

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